Ctenopteryx sicula, a bathypelagic loliginid squid?

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Abstract: *Ctenopteryx sicula* (Vérany, 1851) is an open-eyed, deep-water squid, and as such has traditionally been classified in the suborder Oegopsida (family Ctenopterygidae) along with other families of squid exhibiting these characteristics. *C. sicula* however displays numerous morphological features, including fused axons in the giant nerve fiber system and accessory nidamental glands, found otherwise only within members of the myopsid families Loliginidae and Pickfordiateuthidae. This has lead previous authors to suggest that *Ctenopteryx* species would be more appropriately placed in the suborder Myopsida. Here biochemical genetic evidence is presented which indicates that *C. sicula* is more closely related to several loliginid species than to species of the oegopsid families Histioteuthidae, Ommastrephidae, and Enoploteuthidae. These data, in conjunction with new data on comparative beak morphology, also suggest that *C. sicula* should be considered an oceanic myopsid species.

The squids (Teuthoidea) are generally considered to be divided into two suborders: the Oegopsida which are mainly oceanic, open-water species which lack a corneal membrance covering the eye, and the Myopsida which are mainly near-shore species and which posses a corneal membrane. This membrane has been described as an adaptation preventing ocular damage in shallow, sediment-rich waters of continental shelf areas (Morton and Yonge, 1964). *Ctenopteryx sicula* (Vérany, 1851) (family Ctenopterygidae) is a bathypelagic squid species of cosmopolitan oceanic distribution (Nesis, 1987; Roeleveld *et al.*, 1992). The species lacks an eye-covering corneal membrane and as such is classified in the suborder Oegopsida along with other families of squid exhibiting this characteristic (for example, see Roper *et al.*, 1969; Nesis, 1987). Naef (1923) and Young (1991) have however highlighted a number of morpological features which are at odds with this scheme of classification. *C. sicula* exhibits fusion of nerve axons in the giant fiber system, and has accessory nidamental glands, features which are otherwise found exclusively within the myopsid families Loliginidae and Pickfordiateuthidae. In addition, *C. sicula* has attachments of the fourth buccal connectives similar to those of *Loligo* species, and has suckers on the buccal lappets, exhibits retraction of tentacles into pockets, and has straight, simple funnel locking cartilages, all of which are reminiscent of loliginid morphology (Naef, 1923; Nesis, 1987; Young, 1991). Young (1991) consequently suggested that *C. sicula* could be a squid species related to the loliginids which has become adapted to life in deep water. Here biochemical genetic techniques are used to investigate relationships between *C. sicula* and member species of the families Loliginidae, Histioteuthidae, Ommastrephidae, and Enoploteuthidae in an attempt to determine to which families it is most closely related, and hence with which suborder the species has closet affinity.

Enzyme electrophoresis is well established as a tool for investigation of taxonomy and systematics (Avise, 1974, 1983; Ferguson, 1980; Ayala, 1983; Richardson *et al.*, 1986; Thorpe and Solé-Cava, 1994), and has been applied to a number of cephalopod problems (Smith *et al.*, 1981; Augustyn and Grant, 1988; Brierley and Thorpe, 1994; Yokowa, 1994; Brierley *et al.*, in press). The theoretical basis for the use of electrophoretic data in taxonomic studies is founded on what has become known as the *molecular clock hypothesis* (Thorpe, 1982; Nei,1987). This hypothesis holds that between reproductively isolated groups of organisms molecules such as enzymes, the structures of which are under direct genetic control, diverge at a rate that is stochastically related to the evolutionary time since divergence. The majority of cases employing electrophoresis as a taxonomic aid have addressed questions raised at the level of populations, species, or genera. The technique has however also provided meaningful data for comparisons between species from related families (for example, see Solé-Cava *et al.*, 1992, 1994), and is therefore an appropriate means for investigation of the question in hand.

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MATERIALS AND METHODS

Squid samples

Ctenopteryx sicula (Vérany, 1851) and the oegopsid squid species *Pyroteuthis margaritifera* (Ruppell, 1844), *Histioteuthis bonellii* (Férussac, 1835), and *Todarodes sagittatus* (Lamarck, 1799) were caught from the northeastern Atlantic Ocean in the region of 21°W, 31°N using a multiple-opening, 8 m^2 rectangular mid-water trawl (RMT8) during RRS *Discovery* cruise 194 in August 1990 (see Herring, 1990). *Sepioteuthis lessoniana* Lesson, 1830, was obtained from Indonesian waters, and *Loligo forbesi* Steenstrup, 1856, and *Loligo vulgaris vulgaris* Lamarck, 1798, were caught around the British Isles. Samples of mantle tissue were dissected from each specimen as soon after capture as possible, and immediately frozen (to -80°C in the case of the *Discovery* samples) prior to transportation to Port Erin Marine Laboratory. Date and location of capture, and species sample sizes, are presented in Table 1.

Electrophoresis

Mantle tissue samples from *Ctenopteryx sicula* and the other oegopsid species were assayed during September 1990 using a series of standard electrophoretic techniques which have been described in detail elsewhere (Brierley, 1992; Brierley *et al.*, 1993a, b). The majority of the loliginids had been examined previously using the same experimental protocols, but a group of ten were run again concurrently with the *Discovery* samples in order to provide a reference for relative band migration. Gels were scored immediately after optimum stain development, and genotypes assigned accordingly.

Data analysis

Allele frequency data was analyzed using the FORTRAN program BIOSYS-1 (Release 1.7) (Swofford and Selander, 1981) to calculate unbiased genetic identity *(I)* and distance *(D)* (Nei, 1978) between species pairs, and to construct a dendrogram of *D* using the unweighted pair-grouping arithmetic mean (UPGMA) cluster analysis algorithm (Sneath and Sokal, 1973). The use of the UPGMA method in conjunction with *D* is generally recognized as the best and most accurate procedure for phylogentic tree reconstruction from electrophoretic data (Nei, 1987). This is partly because *D* can be subject to large stochastic errors (especially if calculated with small numbers of loci), and the procedure of distance-averaging used in UPGMA reduces this error considerably (Nei, 1987).

RESULTS

Seventeen enzyme stain systems developed successfully for all species, revealing the presence of 18 common putative enzyme loci. The group of ten loliginids run concurrently with *Ctenopteryx sicula* and the oegopsid species resolved identically here as previously (Brierley *et al.*, 1993a, 1995; Brierley and Thorpe, 1994). A slight reduction in band intensity was apparent, but band migration and definition remained entirely unaffected by frozen (-26°C) storage. Allele frequencies at the 18 clearly resolving enzyme loci are given for each species in Table 2. Inspection of Table 2 reveals *Histioteuthis bonellii*, *Todarodes sagittatus*, and *Pyroteuthis margaritifera* exhibiting very low levels of mean heterozygosity per locus, a phenomemon which is a seemingly recurrent theme in studies of squid biochemical genetics (see review in Brierley *et al.*, 1993a). Although this ubiquitous feature of low intraspecific genetic variability can cause problems for the use of electrophoretic data in squid population studies (see Brierley *et al.*, 1993b, 1995), taxonomic studies benefit because banding patterns remain simple, aiding interpretation and greatly reducing the likelihood of error (Garthwaite *et al.*, 1989). Mean *I* and *D* between all species pairs are given in Table 3. These unbiased estimates are most appropriate for small sample sizes, especially, as here, when observed heterozygosity levels are low. *D* is purported to be stochastically linear with evolutionary time (Nei, 1987; Thorpe, 1989), and varies between a theoretical minimum of zero in comparison of genetically identical individ-

Table 1. Sample size, and date and location of capture of species examined.

Species	Sample size	Date of capture	Location of capture
Ctenopteryx sicula	4	August 1990	Northeastern Atlantic Ocean
Pyroteuthis margaritifera	11	August 1990	Northeastern Atlantic Ocean
Histioteuthis bonellii	14	August 1990	Northeastern Atlantic Ocean
Todarodes sagittatus	2	August 1990	Northeastern Atlatnic Ocean
Sepioteuthis lessoniana	5	April 1988	South Alas Strait, Indonesia
Loligo forbesi	20	October 1989	Isle of Man, British Isles
L. vulgaris vulgaris	20	October 1989	Plymouth, United Kingdom

Table 2. Allele frequencies at the 18 clearly resolving enzyme loci.

Locus	Allele	*Ctenopteryx sicula* N = 4	*Loligo forbesi* N = 20	*L. v. vulgaris* N = 20	*Sepioteuthis lessoniana* N = 5	*Pyroteuthis margaritifera* N = 11	*Histioteuthis bonnellii* N = 14	*Todarodes sagittatus* N = 2
aGpdh	A	0	0	0	0	0	1	0
	B	0.125	0	0	0	0	0	0
	C	0.875	0	0	0	0	0	1
	D	0	0	0.9	0	0	0	0
	E	0	1	0.1	0	0	0	0
	F	0	0	0	1	1	0	0
Mdh- 1	A	0	1	1	0	0	0	0
	B	0	0	0	1	0	0	0
	C	0.875	0	0	0	0	0	0
	D	0	0	0	0	1	0	1
	E	0.125	0	0	0	0	1	0
Mdh-2	A	0	0	0	0	0	1	0
	B	1	0	0	1	0	0	0
	C	0	1	1	0	0	0	0
	D	0	0	0	0	0	0	1
	E	0	0	0	0	1	0	0
Me	A	0	0	0	1	0	0	0
	B	0	1	1	0	0	0	0
	C	0	0	0	0	1	0	0
	D	1	0	0	0	0	0	0
	E	0	0	0	0	0	1	0
	F	0	0	0	0	0	0	1
Idh	A	0	0	0	0	0	0	1
	B	0	0	0	0	0	1	0
	C	1	0	0	1	0	0	0
	D	0	0.95	1	0	0	0	0
	E	0	0.05	0	0	1	0	0
Pgdh	A	0	0	0	1	0	0	0
	B	0	0	1	0	0	0	0
	C	0	0.025	0	0	0	0	0
	D	0	0.975	0	0	0	0	0
	E	0	0	0	0	1	0	0
	F	1	0	0	0	0	0	0
	G	0	0	0	0	0	1	0
	H	0	0	0	0	0	0	1
Gopdh	A	0	0	0	0	1	0	0
	B	0	0	0	0	0	0	1
	C	0	1	0	1	0	0	0
	D	0	0	1	0	0	0	0
	E	0	0	0	0	0	1	0
	F	1	0	0	0	0	0	0
Gapdh	A	0	0	0	0	1	0	0
	B	0	0	0	0	0	0	1
	C	0	1	0	0	0	0	0
	D	0	0	0	1	0	0	0
	E	1	0	1	0	0	0	0
	F	0	0	0	0	0	1	0
Sdh	A	0	0	0	0	0	1	0
	B	0	0	0	0	1	0	0
	C	1	0	0	0	0	0	0
	D	0	1	0	0	0	0	0
	E	0	0	0	1	0	0	1
	F	0	0	1	0	0	0	0
Pep-A	A	1	0	0	0	0	0	0
	B	0	1	0	0	0	0	0
	C	0	0	1	0	0	0	0
	D	0	0	0	0	0	0	1
	E	0	0	0	1	0	0	0
	F	0	0	0	0	1	0	0
	G	0	0	0	0	0	1	0

(Continued)

Table 2. Continued.

Locus	Allele	*Ctenopteryx sicula* N = 4	*Loligo forbesi* N = 20	*L. v. vulgaris* N = 20	*Sepioteuthis lessoniana* N = 5	*Pyroteuthis margaritifera* N = 11	*Histioteuthis bonnellii* N = 14	*Todarodes sagittatus* N = 2
Ck	A	0.125	0	0	0	0	0	0
	B	0.875	0	0	0	0	0	0
	C	0	1	1	0	0	0	0
	D	0	0	0	1	0	0	0
	E	0	0	0	0	1	1	1
Fum	A	0	0	0	0	0	0	1
	B	0	1	0	0.5	0	0	0
	C	1	0	0	0	0	0	0
	D	0	0	0	0	1	0	0
	E	0	0	1	0	0	0	0
	F	0	0	0	0.5	0	0	0
	G	0	0	0	0	0	1	0
Mpi	A	0	0	0	0.5	0	0	0
	B	0	0	0	0	0	0	1
	C	0	0	0	0.5	0	0	0
	D	0	0	1	0	0	0	0
	E	1	0.975	0	0	0	0	0
	F	0	0.025	0	0	1	1	0
Gpi	A	0	0	0	0	0	0	1
	B	0	0	0	0	1	0	0
	C	1	0	0	0	0	0	0
	D	0	1	1	0	0	0	0
	E	0	0	0	1	0	0	0
	F	0	0	0	0	0	1	0
Ald	A	0	1	1	1	0	1	1
	B	1	0	0	0	0	0	0
	C	0	0	0	0	1	0	0
Odh	A	0	1	0	1	0	0	0
	B	1	0	1	0	1	0	0
	C	0	0	0	0	0	1	0
	D	0	0	0	0	0	0	1
Ldh	A	1	0	0	0	0	0	0
	B	0	1	0.825	0	0	0	0
	C	0	0	0	0	1	0	0
	D	0	0	0	1	0	0	0
	E	0	0	0.175	0	0	0	0
	F	0	0	0	0	0	1	0
	G	0	0	0	0	0	0	1
Sordh	A	0	0.125	0	0	0	0	0
	B	0	0.85	0	1	0	0	0
	C	0	0.025	0	0	0	0	0
	D	0	0	1	0	0	0	0
	E	1	0	0	0	0	0	0
	F	0	0	0	0	1	0	0
	G	0	0	0	0	0	0	1
	H	0	0	0	0	0	1	0

Table 3. Matrix of unbiased genetic identity *(I)* (above diagonal) and distance *(D)* (below diagonal) values between all species pairs.

Species	1	2	3	4	5	6	7
1 *Ctenopteryx sicula*	****	0.056	0.115	0.117	0.057	0.007	0.05
2 *Loligo forbesi*	2.881	****	0.449	0.253	0.004	0.058	0.056
3 *L. vulgaris vulgaris*	2.162	0.800	****	0.058	0.056	0.056	0.056
4 *Sepioteuthis lessoniana*	2.144	1.375	2.845	****	0.057	0.057	0.115
5 *Pyroteuthis margaritifera*	2.869	5.468	2.877	2.859	****	0.111	0.111
6 *Histioteuthis bonnellii*	4.949	2.853	2.877	2.859	2.197	****	0.111
7 *Todarodes sagittatus*	3.003	2.877	2.877	2.165	2.197	2.197	****

uals, and infinity when the pair of taxa under consideration exhibits no common genetic characters. A UPGMA dendogram of *D* (Fig. 1) shows *Ctenopteryx sicula* clustering more closely with the branch encompassing the loliginid species than to the branch containing the oegopsid families Histioteuthidae, Ommastrephidae, and Enoploteuthidae.

DISCUSSION

In contrast to electrophoretic studies of population structuring, in which large sample sizes are required, taxonomic studies such as the one reported here can be successfully conducted using only very small numbers of individuals from each taxa if large numbers of enzyme loci are studied (see for example Richardson *et al.*, 1986). The comparatively small sample sizes of *Ctenopteryx sicula, Todarodes sagittatus,* and *Sepioteuthis lessoniana* are unlikely to be a major source of inaccuracy in calculation of *D*, or, consequently, in reconstruction of the phylogenetic tree shown in Fig. 1. Numbers of animals used have negligible effects upon the errors of genetic distance, and even sample sizes as small as one will give acceptable distance estimates (see Nei, 1978; Gorman and Renzi, 1979; Thorpe, 1982). This fact is well illustrated by a reconstructed phylogeny of the family Ommastrephidae (Yokowa, 1994) which is based on electrophoretic data gathered from 16 species with a mean sample size of less than two.

Yeatman and Benzie (1993) used *Sepioteuthis lessoniana* as an outgroup in a study of cryptic speciation in *Loligo* from Australian waters. They reported the species differing completely from all *Loligo* taxa investigated at eight of the 11 loci they resolved, and consequently reported a *D* value of > 4 between species. Such a high value is far larger than would normally be expected between members of confamilial genera (Thorpe, 1982). We have assayed six (*Ak, Gpi, Idh, Mdh-1, Mdh-2,* and *Mpi*) of these eight loci here and elsewhere (Brierley, 1992; Brierley *et al.*, 1993b, in press) and similarly find alleles unique to *Sepiotheuthis lessoniana* at these loci. However this and our previous analyses have included data from a number of other loci in addition to the 11 investigated by Yeatman and Benzi (1993), and our estimate of *D* between *Loligo* species and *Sepioteuthis lessoniana* of the order of 1.4 is therefore likely to be the more accurate. This large discrepancy between studies highlights the possibility of substantial interlocus errors on measures of genetic distance (Thorpe, 1979, 1982), and emphasizes the importance of screening large numbers of loci.

The phylogenetic tree reconstructed using UPGMA analysis of *D* between all species pairs (Fig. 1) comprises two main branches, one containing member species of the suborder Oegopsida, and the other containing myopsid species. *Ctenopteryx sicula* clusters more closely with the Myopsida, and genetic data presented here are therefore in agreement with morphological evidence (Naef, 1923; Young, 1991) suggesting the species is related to *Loligo. C. sicula* clusters with other myopsid species at a level of *D* which lies within the practical limits of the measure. The *D* value of about 2.9 exhibited here between *C. sicula* and *Loligo forbesi* (see Table 3) is less than the value of $D = 3$, beyond which the measure diverges from linearity (Thorpe, 1989). This limit arises because *D* appears to suffer a "saturation" effect (Thorpe, 1982): a maximum of one nucleotide substitution per locus can be detected electrophoretically, and practical limitations impose a resolution threshold on

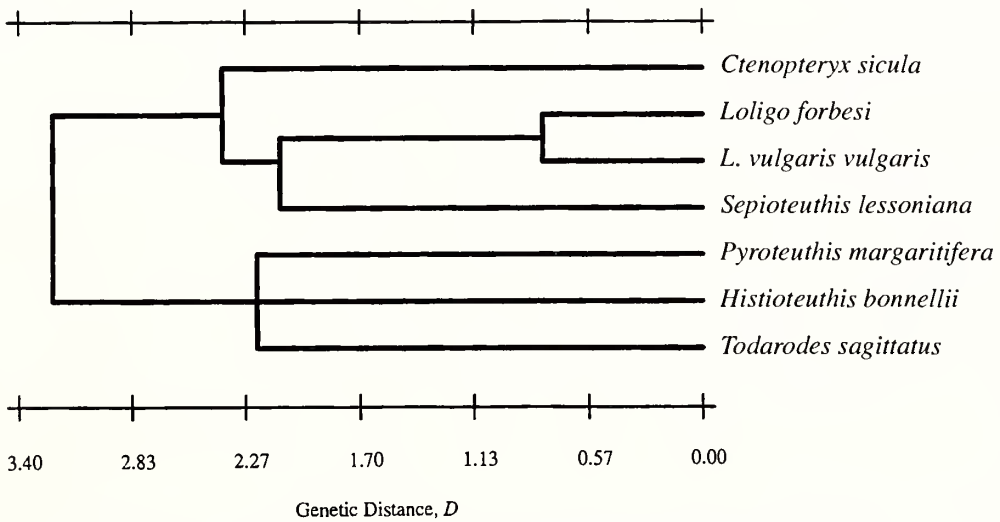

Fig. 1. UPGMA dendrogram of Nei's (1978) unbiased genetic distance *(D)* between all species pairs.

the number of discrete alleles which can be electrophoretically distinguished. Using data gathered from a survey of around 100 loci between species pairs from different major taxa, Thorpe (1982) found an approximate 5% coincidental identity between quite unrelated taxa (I in the order of 0.05, $D = -\log_e I$). The similarity between *C. sicula* and the loliginid species studied here is clearly greater than this 5% similarity. The level at which the oegospid and myopsid species cluster is high, but this does not detract from the validity of the observation that members of the Myopsida and Oegopsida cluster as two discrete entities, or that *C. sicula* is more closely related to loliginids than to any of the oegospid families studied here. We do not attempt to place an exact time of divergence on any of the branch points in Fig. 1, because the absolute relationship between genetic distance and evolutionary time is questionable (Sarich, 1977; Lessios, 1979; Thorpe, 1982; Smith and Coss, 1984). For most taxonomic purposes however, as here, the problem of absolute nonlinearity of D with time is unimportant because only relative values are needed (Thorpe, 1982). As long as genetic distance is approximately linear with time, the relative evolutionary relationships among organisms can be determined (Nei,1987).

Ctenopteryx sicula is positioned within the branch of the phylogenetic tree (Fig. 1) containing *Loligo forbesi* and *L. vulgaris vulgaris* beyond the junction at which *Sepioteuthis lessoniana* diverges from the genus *Loligo*. *Sepioteuthis* has been described, on morphological and distributional grounds, as a Tethyan relict genus which split off from all other loliginids before the closing of the Tethys Sea (Brakoniecki, 1986). *C. sicula* would therefore appear to have diverged from the loliginid line at some time before this event. Naef (1923) suggested that *C. sicula* should be considered the earliest independent branch of the stem of the higher Oegopsida. Genetic data from the present study summarized in the phenogram in Fig. 1 go some way to support this assertion, although further electrophoretic analysis of *Pickfordiateuthis pulchella* Voss, 1953, would be necessary before genetic and morphological data could be viewed in complete congruence. It would seem appropriate therefore to adopt the viewpoint of Young (1991), who has proposed that *C. sicula* may be considered as a teuthoid related to loliginids which has become adapted to a deep-water, oceanic life. Young (1991) has also argued that, despite the fact that the name is inappropriate for a squid exhibiting the open-eyed condition, it would be desirable to classify *Ctenopteryx* species with the families Loliginidae and Pickfordiateuthidae in the suborder Myopsida.

The numerous similarities between *Ctenopteryx* and *Bathyteuthis* species have been well described (Roper, 1969; Nesis, 1987), and more recent analyses of comparative beak morphology have further highlighted the affinity among *Loligo, Ctenopteryx,* and *Bathyteuthis* species (Roper and Clarke, unpub. data). It would be of additional interest to examine *Bathyteuthis* species electrophoretically, as it remains possible that these species similarly fail to comply with the broad categorization of an open-eyed, open-ocean suborder, contrasting entirely with a distantly related shelf-inhabiting group. Our data suggest that the character of the presence or absence of a corneal membrane may not be an infallible guide for distinguishing the two major systematic groupings of squid.

ACKNOWLEDGMENTS

We should like to thank Dr. Peter Herring for providing the opportunity to collect samples during RRS *Discovery* cruise 194, and for invaluable help with squid identification at sea. We also thank the scientists, Captain and crew of RRS *Discovery* cruise 194, the Captain and crew of Liverpool University's RV *Cuma*, Dr. A. Ghofar and Mr. P. Pascoe for assistance with sample collection, and Professor T. Norton for provision of laboratory facilities at Port Erin Marine Laboratory. This study was supported by grant number GR3/6849 awarded by the Natural Environment Research Council to Dr. J. P. Thorpe.

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Date of manuscript acceptance: 5 February 1996